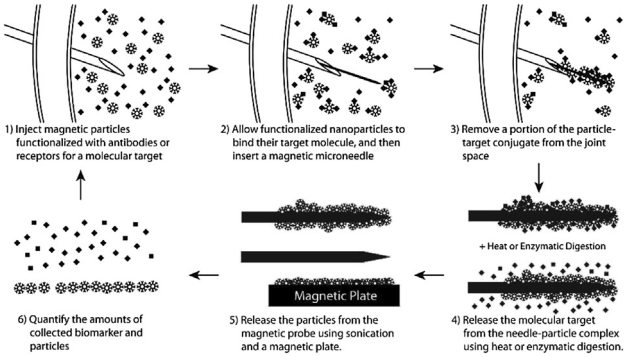
hindered by inability to collect sufficient synovial fluid via joint aspiration. Lavage and fluid wicking can acquire synovial fluid samples in small animals; however, both techniques are primarily used following animal euthanasia, making longitudinal OA biomarker analysis practically impossible in a small animal model.

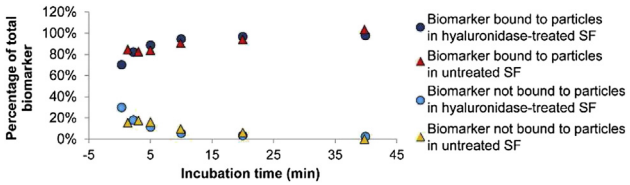
Our group has developed a magnetic nanoparticle-based technology to collect OA biomarkers from synovial fluid (Figure 1). First, an antibody against an OA biomarker is conjugated to the surface of magnetic particles. Antibody-conjugated particles are used to bind biomarkers in synovial fluid. Then, a magnetic probe is used to collect a percentage of the particle-biomarker conjugate from synovial fluid. Biomarker is released from particles using heat, then particles are released from the probe using sonication and a magnetic plate. Using a biomarker collected per particle ratio, the amount of biomarker in synovial fluid can be estimated. The purpose of this study is to assess the sensitivity of magnetic collection relative to ELISAs conducted on small volume synovial fluid samples.

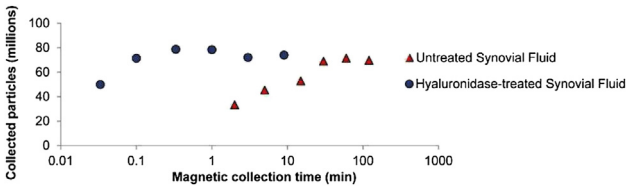
Methods: Antibodies against a widely-investigated OA biomarker, C-telopeptide of type II collagen (CTXII, Immunodiagnostic Systems), were conjugated to 1-2 μm diameter polystyrene particles containing 10-20 nm diameter superparamagnetic iron oxide nanoparticles (SPIONS) in the particle core (10% SPIONS by volume). The effect of synovial fluid viscosity on the magnetic collection technique was investigated by artificially degenerating synovial fluid with hyaluronidase (0.01 mg/ml for 4.5 hr at room temperature). In particular, 1 billion particles with approximately 400 antibody molecules per particle were mixed with 400 μL of hyaluronidase-treated or untreated bovine synovial fluid. To investigate the antibody-CTXII binding kinetics, 50 μL aliquots were acquired for analysis at approximately 1, 2.5, 5, 10, 20, and 40 min. In addition, magnetic particles with approximately 800 antibody molecules per particle were mixed with either hyaluronidase-treated or untreated bovine synovial fluid (SF), subjected to constant gentle mixing for 1 h, then divided into 25 μL samples (97 million particles per sample). In these samples, magnetic needles were inserted for varying time intervals and the amount of collected particles was determined by fluorescence.

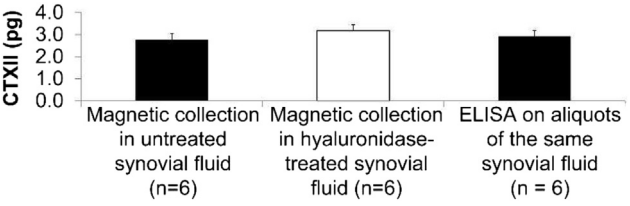
Results: Synovial fluid viscosity had minor only effects on antibody-biomarker binding kinetics (Figure 2), indicating the ratio of biomarker to antibody (or biomarker per particle) could be used to estimate the initial biomarker amount in a sample as long as magnetic collection was conducted after a dynamic equilibrium was been established. Synovial fluid viscosity did have marked effects on the collection efficiency of magnetic particles in synovial fluid (Figure 3). However, since magnetic collection occurred only after the antibody and biomarker reached a dynamic binding equilibrium, the ratio of biomarker to antibody was able to estimate the initial concentration of CTXII in a synovial fluid sample with similar precious to an ELISA conducted on an aliquot of the same synovial fluid sample (Figure 4).

Conclusions: In this work, critical variables for magnetic collection of CTXII are described, thereby demonstrating the potential to magnetically collect OA biomarkers in synovial fluid volumes similar to that of a rat knee. Future work will focus on demonstrating the technique in an intact rat knee, where joint geometry is far more complex than the present in vitro studies. Nonetheless, these data demonstrate an in vitro proof-of-concept for magnetic collection of OA biomarkers.









117
HIGHER SERUM LEVELS OF CARTILAGE OLIGOMERIC MATRIX PROTEIN (COMP) ARE ASSOCIATED WITH SELF-REPORTED KNEE PAIN

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Purpose: Intermittent knee pain is a prevalent complaint in the middle-aged population but its significance is poorly understood in the absence of definitive structural changes. Increased levels of cartilage oligomeric matrix protein (COMP), a potential prognostic biomarker in early knee OA, have been associated with risk of incident radiographic knee OA. In this study, we looked at the cross-sectional association of serum COMP levels and self-reported knee pain in middle-aged UK females.

Methods: We looked at 724 subjects from the Chingford Cohort Longitudinal Study who had serum levels of COMP measured at year 2 and 3, and data on self-reported knee pain at year 3. Knee pain was defined as 'any knee pain' if it was present on any number of days in the preceding month in either knee and 'pain on most days' if it was present in either knee on most days in the preceding month. COMP levels were categorised into quintiles. A logistic regression model was utilised to investigate associations between the presence of the knee pain at year 3 and serum COMP levels.

Results: Mean age was 55 years (SD +/- 5.9; Range 46-68), Mean BMI was 25.3 (SD +/- 4; Range 17-47). 205 out of 724 subjects reported 'any knee pain' in the last month, and 80 reported 'pain on most days' of the preceding month. The highest COMP quintile (levels above 12.63 U/L), compared to the lowest, was significantly associated with 'any knee pain' in the last month (OR 1.92, 95% CI 1.09 – 3.23). This association remained significant after adjusting for age and vitamin D levels (OR 1.89, 95% CI 1.14 – 3.27), but not after adjustment for BMI (OR 0.7, 95%CI 0.3 – 1.52).

Conclusions: Higher levels of serum COMP are associated with 'any knee pain', but not with reporting knee 'pain on most days' in the preceding month. High COMP levels are associated with intra-articular joint changes within synovium, cartilage and ligaments. Considering that inflamed synovium is a significant pain generator, episodic knee pain associated with high COMP levels may reflect low-grade synovial inflammation.